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Learning and Memory Enhancement by Neuropeptides
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The major purpose of this work, is to study mechanisms responsible for the toxic effects of the organometal neurotoxin trimethyltin (TMT) on learning, in order to develop strategies for prevention or alleviation of toxicity. Trialkyltins are used as stabilizers for plastics, or as biocides for control of fungus, barnacles, bacteria and insects. Their toxic effects have been known for over 100 years (1). However, the specific neurotoxic effects of TMT were first observed after accidental exposure of two French chemical workers who experienced memory loss and seizures (2). Since then, numerous investigations in rodents have confirmed that TMT, administered systemically, produces a relatively specific lesion in hippocampus and related olfactory cortical structures (3). These lesions are associated with impairments in learning and memory as measured in a wide variety of tasks (4). Thus, as well as being an environmental anti-fouling toxicant of specific interest to the Navy, the compound may also be of interest as a model treatment for study of learning/memory dysfunction resulting from exposure to other toxicants (e.g. other heavy metals, organic solvents), or arising from disease states.

We study learning in an autoshaping task, in which rats learn to touch a lever to obtain food (5). During the past year we have completed an initial dose-response study of effects of TMT on autoshaping and a paper has been accepted for publication (C.A. Cohen, R.B. Messing and S.B. Sparber, Psychopharmacology, in press). This study separates "non-specific" behavioral changes which may affect behavior of toxicant-exposed rats in assays of learning or memory from specific cognitive effects of the compound. In particular, TMT does not impair performance of easy versions of the autoshaping task with identical sensorimotor and motivational requirements, but when a delay of reinforcement of 6 sec is imposed rats given all three doses (3, 6 or 7.5 mg/kg of TMT) show impaired performance. However, the high dose rats show hyperreactivity and perseverative behavior (similarly to rats with large conventional lesions of the hippocampus), and actually manipulate the lever more than rats given lower doses. These high dose rats fail to learn a latent inhibition paradigm, also similarly to rats with large hippocampal lesions. The results thus suggest that the cognitive impairment may be separable from hyperreactivity and perseveration, since the latter effects only emerge at the high dose. Our low dose rats appear to be similar to normally aged rats, since they learn a task analagous to a classical conditioning delay paradigm, but cannot learn a trace paradigm (6). We have previously shown that rats treated with TMT have increased concentrations of forebrain ß-adrenergic receptors, and have hypothesized that these rats may have a deficiency of forebrain norepinephrine release similar to that seen with aged animals (7).



Also within the past year, we have completed studies showing that rats treated with TMT or a mixed ganglioside preparation (which was administered to determine a possible therapeutic effect in TMT-treated animals) have decreased concentrations of hippocampal glucocorticoid receptors, which may be related to cognitive impairments. This is another parallel between these animals and aged rats which have deficiencies in this receptor (8). Complete manuscripts for this work are in preparation, but some of the work will be presented at the Xth International Congress of Pharmacology in August. Interestingly, TMT-treated rats have elevated levels of glial fibrillary acidic protein (GFAP), an indication of the cytotoxicity produced by this compound. Rats treated with gangliosides, which induce a cognitive impairment but no cell death, have normal levels of GFAP, but still exhibit the decrease in corticosteroid binding. Thus, this decrease is

probably independent of hippocampal cell death, and may be a down regulation. In future work we wish to examine the effects of manipulations of the pituitary adrenal axis on TMT toxicity as measured behaviorally, biochemically and histologically.

We have also completed a study of the effects of the $\alpha_2$-noradrenergic receptor antagonist yohimbine on autoshaping (M.S. Huang, R.B. Messing and S.B. Sparber, Life Sciences [1987] 41: 1083-1088). This prototypical anxiogenic agent, which increases forebrain norepinephrine release, enhances autoshaping in low doses, as does the putative learning/memory enhancing peptide, des-glycinamide arginine-8-vasopressin (DGAVP), as demonstrated in previous work (9-11). However, unlike DGAVP, yohimbine also induces increased behavioral arousal, which may account for its effects on autoshaping. In view of the altered norepinephrine receptor binding in forebrain of TMT-treated rats, we plan to test yohimbine and related compounds in rats treated with TMT. Within the year, we have completed studies showing that DGAVP attenuates the learning impairment in TMT-treated animals, and that TMT induces a specific learning deficit without a retrieval deficit: rats which have already learned the autoshaping task and given TMT continue to perform. The manuscript describing this work has been submitted for publication, and an abstract will be presented at the Neuroscience meeting in New Orleans (Society for Neuroscience Abstracts, in press). We have also done some work with the opiate antagonist naloxone, a substance which has memory-enhancing properties in many assays, but have found that this substance actually impairs acquisition of autoshaped behavior, whether given before or after training sessions. A manuscript describing this work is in preparation, and an abstract will be presented at the Neuroscience meeting in New Orleans (Society for Neuroscience Abstracts, in press).

We have also demonstrated, within the last year, that autoshaping is highly dependent upon the deprivation state of the animal: more food deprived rats learn faster. This is not simply a generalized behavioral activitation produced by food deprivation, since more food deprived rats also show better learning of latent inhibiiton. It appears that deprivation state also influences the performance of rats exposed to TMT, similarly to its effect in normal animals. This is consistent with the capacity for DGAVP to attenuate the learning deficit in rats treated with TMT, and indicates that the learning impairment is not absolute, but rather may be amenable to various palliative treatments. A manuscript describing this work is also in preparation. Our present research plans include studies investigating interactions between deprivation levels and effects of drugs and toxicants.

Finally, work in progress at the moment, is concerned with two issues:

1. We have found that 6.0 mg/kg of TMT induces a decrease in hippocampal corticosterone receptors (see above). However, a higher dose (7.5 mg/kg) does not induce as large a decrease (in parallel with behavioral data showing that these animals make more lever touches in our autoshaping task) (see above). When rats given 6.0 mg/kg of TMT are required to respond in a n operant fixed ratio paradigm in which the ratio requirement (number of responses necessary to obtain a food pellet) is doubled each day (progressive fixed ratio task), rats given 6.0 mg/kg make fewer responses than controls as the ratio requirement increases, while rats given 7.5 mg/kg of TMT make more responses than controls. Thus, rats given 6.0 mg/kg of TMT appear to provide a convenient preparation with which to investigate the functioning of hippocampal steroid receptors.

2. We have also been investigating the seemingly paradoxical effect of the high dose of TMT in our behavioral and biochemical assays. (These animals sustain the largest amount of tissue loss, as measured by hippocampal weights, despite their apparently more "normal" biochemistries). Current behavioral research is focusing on the strength of conditioning which occurs in these animals. It appears that although they may be hyperreactive to the lever, these animals do not behave similarly to normal animals during intertrial or reinforcement delay intervals. Normal animals spend more time near the lever

during intertrial intervals, and more time near the trough during reinforcement delays. In future work, we hope to find a biochemical correlate for these animals, and initial studies will be on glutamate receptors.

## References

1. Jolyet, F. & Cahours, A. (1869) C.R. de l'Acad. Sci. (Paris) 68: 1276.
2. Fortemps, E. et al. (1978) Int. Arch. Occup. Environ. Hlth. 41: 1.
3. Chang, L.W. & Dyer, R.S. (1985) Neurobehav. Toxicol. Teratol. 7: 43.
4. Messing, R.B. (1987) In: H.A. Tilson & S.B. Sparber (Eds.) Neurotoxicants and Neurobiological Function, New York: John Wiley & Sons, pp. 279-302.
5. Graves, C.A. & Solomon, P.R. (1985) Behav. Neurosci. 99: 88-96.
6. Messing, R.B. In: G. Stefano (Ed.) Handbook of Comparative Opioid and Related Neuropeptide Mechanisms, Boca Raton, FL: CRC Press, pp. 259-270.
6. Messing, R.B. & Sparber, S.B. (1986) Toxicol. Lett. 32: 107.
7. Sapolsky, R.L. et al. (1983) Brain Res. 289: 235.
8. Messing, R.B. & Sparber, S.B. (1983) Eur. J. Pharmacol. 89: 43.
9. Messing, R.B. & Sparber, S.B. (1984) Trends in Pharmacol. Sci. 5: 149.
10. Messing, R.B. & Sparber, S.B. (1985) Behav. Neurosci. 99: 1114.

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M.S. Huang, R.B. Messing and S.B. Sparber, Learning Enhancement and Behavioral Arousal Induced by an Anxiogenic Drug, Life Sciences [1987] 41: 1083-1088.
A COPY OF THIS REPRINT IS APPENDED TO THIS LETTER.

C.A. Cohen, R.B. Messing and S.B. Sparber, Selective Learning Impairment of Delayed Reinforcement Autoshaped Behavior Caused by Low Doses of Trimethyltin, Psychopharmacology, in press.

S.B. Sparber, C.A. Cohen and R.B. Messing, Reversal of a trimethyltin-induced learning deficit by desglycinamide-8-arginine vasopressin, submitted to Life Sciences.

S.B. Sparber, G. Seran, R.B. Messing, J.O'Callaghan and B. Berra, Toxicity of mixed gangliosides (GS): Learning and Hippocampal Corticosterone Receptors. Xth International Congress of Pharmacology Abstracts, in press.

R.B. Messing, S.Allen, L. Aanonsen and S.B. Sparber, Post-session naloxone administration impairs autoshaped lever response learning. Society for Neuroscience Abstracts, in press.

S.B. Sparber, C.A. Cohen and R.B. Messing, Desglycinamide-8-arginine vasopressin reverses a trimethyltin-induced learning deficit. Society for Neuroscience Abstracts, in press.

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# LEARNING ENHANCEMENT AND BEHAVIORAL AROUSAL INDUCED BY YOHIMBINE[1]

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## Summary

Learning of a food motivated delayed reinforcement autoshaping task was investigated in rats treated with water vehicle or the prototypical anxiogenic agent and $\alpha_2$-adrenergic antagonist yohimbine (0.5 or 1.5 mg/kg, i.p. 30 min before behavioral testing). Unconditioned exploratory rearing activity was monitored concomitantly with acquisition of a lever touch response. The low dose of yohimbine enhanced learning, but it also increased unconditioned behavioral arousal. The high dose retarded acquisition, but when it was withdrawn the animals learned but exploratory activity increased beyond control levels prior to acquisition. Learning thus appeared to be related to the behavioral arousal produced by yohimbine, suggesting that learning enhancement by anxiogenic substances is not due to a direct effect on processes intrinsic to information storage and retrieval; rather, anxiogenic substances may be important modulators of vigilance and performance variables.

Clinical and experimental findings indicate that anxiolytic drugs (e.g. benzodiazepines) impair performance in learning and memory tasks (1-5). However, the preponderance of evidence suggests that these effects are most probably the result of sedative and related properties of these agents, which affect sensorimotor and motivational variables necessary for learning and performance, rather than of direct action on information storage and retrieval. Conversely, a recent study (6) found that an anxiogenic inverse agonist at benzodiazepine receptors, β-carboline-3-carboxylate, enhances performance in learning and memory tasks, an effect the authors hypothesized might be due to an increase in behavioral arousal. We now show that yohimbine, a prototypical anxiogenic agent (7,8) and $\alpha_2$-adrenergic antagonist at low (<1.0 mg/kg) doses (9-11), enhances learning of a delayed reinforcement autoshaping task. However, exploratory rearing activity is also increased as the rats learn. The results suggest that this and other anxiogenic substances that enhance learning may act via effects on arousal processes, rather than on learning or memory storage per se.

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We have previously shown that the putative learning/memory enhancing peptide des-glycinamide arginine vasopressin enhances acquisition of this task without inducing an increase in exploratory activity (12,13). On the other hand, the organometal neurotoxin trimethyltin, which has relatively selective toxic effects on hippocampus and related olfactory cortical structures (14), retards acquisiton of the delayed reinforcement task. Low doses of this toxin, that impair acquisition with a six sec reinforcement delay, have no apparent effect on unconditioned activity, measured during the course of acquisition. Moreover, animals learn easier versions of the task with shorter or no delays, suggesting a specific cognition-impairing effect (15). Thus, the paradigm is sensitive to changes in acquisition performance induced by compounds that selectively enhance or impair learning.

## Materials and Methods

Male Long Evans rats (Blue Spruce Farms, Altamont, NY) initially weighing 410 to 510 g, were deprived to 85-90% of ad lib weights before testing in modified operant chambers. A cue light above a retractable lever remained illuminated throughout behavioral test sessions, and the lever was extended into the chamber according to a random time 45 sec schedule (range of intertrial intervals = 22-68 sec). The lever remained extended either until a touch response was made, or for a maximum of 15 sec. Following a reinforcement delay interval of 6 sec after lever retraction, a 45 mg food pellet (Bioserv, Frenchtown, NJ) was delivered. Food was delivered regardless of whether or not the animal made an extended lever touch (correct response). The task thus incorporates features of both classical and operant conditioning paradigms. Touches of the retracted lever during intertrial and reinforcement delay intervals, which may reflect strength of conditioning (16), were also monitored. Operant chambers contained metal strips 7.5 cm wide mounted 15 cm above the floor on the walls opposite the lever and door. Unconditioned exploratory rearing (strip touching) activity was measured concomitantly with lever touching, using high resistance drinkometer-type circuits between strips or levers and the grid floor of the chamber (16). Animals (n = 7/group) were given water vehicle (1 ml/kg, i.p.) or yohimbine HCl ( 0.5 or 1.5 mg/kg; doses expressed as salt weights) 30 min before the first 6 of 12 behavioral sessions, each of which contained 12 trials (lever presentations).

Data were analyzed by repeated measures ANOVAs followed by 2-tailed Dunnett's t-tests comparing the vehicle and yohimbine-treated groups.

## Results

The low (0.5 mg/kg) dose of yohimbine significantly increased the rate of acquisition of the extended lever touch response, animals given the low dose making more correct responses/12 trials than controls in Sessions 4-6 (Fig. 1). After this, performance of control rats began to increase. In contrast, the high dose of yohimbine (1.5 mg/kg) retarded acquisition of extended lever touching, animals in this group making fewer correct responses than controls in Sessions 8 and 9.

Differences were also seen in intertrial lever touching (Fig. 2). Maximum levels of this behavior are not as constrained by ceiling effects, compared with extended lever touching, and the development of intertrial lever touching generally follows extended lever touching (15). Thus, it was possible to observe further increases in responding towards the lever in late, as well as early sessions. Again, the group given 0.5 mg/kg of yohimbine responded significantly more than controls in Sessions 5, 6, 9 and 11. The latter

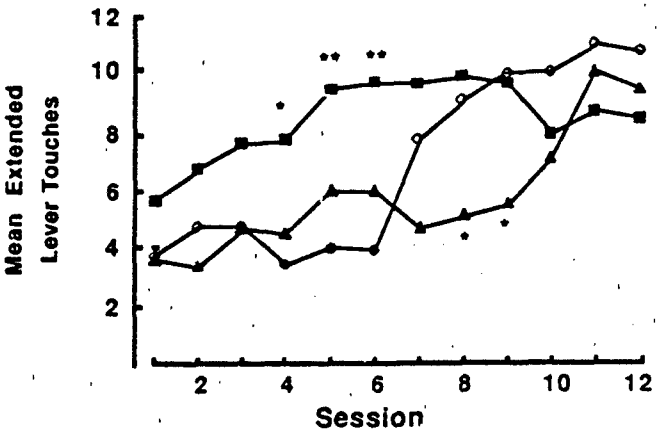


FIG. 1

Effect of yohimbine on extended lever touching (correct responses). Open circles: water; dark squares: 0.5 mg/kg yohimbine; dark triangles: 1.5 mg/kg yohimbine. There was an effect of Session ($F[11,198]=13.5$, $p<.001$) and a Session by Treatment interaction ($F[22,198]=3.9$, $p<.001$). $^{*}p<.05$, $^{**}p<.01$ different from water.

two sessions occurred after drug administration was terminated, indicating that yohimbine did genuinely affect learning, and that performance was not state-dependent: i.e. not dependent upon the presence of yohimbine. No effect of yohimbine was observed on lever touching during reinforcement delays (adventitiously reinforced or superstitious behavior) (not shown).

However, yohimbine also had effects on unconditioned exploratory behavior, that appear to be related to learning (Fig. 3). In early sessions levels of this behavior were high in rats given the low dose of yohimbine. Habituation occurred, and strip touching activity declined to approximately control values around the fourth or fifth session. It is likely that high levels of exploratory behavior facilitate acquisition of lever touching. We have previously observed that females with higher levels of activity than males also acquire lever touch behavior more rapidly (17). In contrast, the high dose group did not show elevated strip touching during treatment. After Session 6, when drug administration was terminated, activity of this group increased above control levels (in Sessions 10 and 11). This could have been due to termination of a suppressant effect of the high dose, and/or to residual, low levels of the drug that directly stimulated strip touching. In any event, as with rats given 0.5 mg/kg, an increase in exploratory behavior preceded acquisition of the extended lever touch response in the high dose group, which was evident in Sessions 11 and 12. Rats in the high dose group also increased intertrial lever touching in later sessions, as extended lever touching increased. This measure increased with session in both the low and high dose groups ($F's[11,198]=4.76$, $p<.001$; 2.93, $p<.005$, respectively) but not in the control group during the course of this experiment.

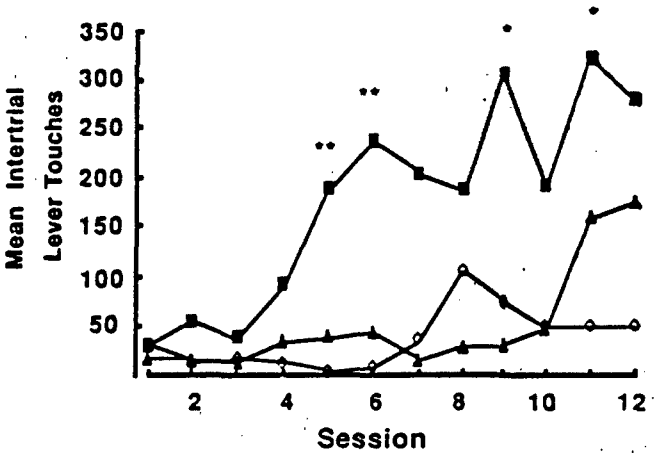


FIG. 2

Effect of yohimbine on conditioned intertrial lever touches. Open circles: water; dark squares: 0.5 mg/kg yohimbine; dark triangles: 1.5 mg/kg yohimbine. There was an effect of Session ($F[11,198]=5.6$, $p<.001$) and a Session by Treatment interaction ($F[22,198]=1.8$, $p<.02$). $^{*}p<.05$, $^{**}p<.01$ different from water.

## Discussion

The results suggest that the enhancement of learning induced by the low dose of yohimbine may be related to behavioral arousal induced by the drug. Similarly to inverse benzodiazepine receptor agonists (18), yohimbine induces a constellation of peripheral autonomic signs associated with stress or arousal, along with increases in subjective anxiety in humans, which can be antagonized by diazepam. Thus, it has been hypothesized that nonadrenergic hyperactivity, which is caused by yohimbine, may be involved in the etiology of anxiety (7,8,10). In this context, it is of interest to note that direct stimulation of the locus coeruleus in animals is associated with increases in behavior which have been likened to fear (19). Studies with animals have found both locomotor excitatory and depressant effects of yohimbine (10). The present results suggest that arousing effects of the drug are probably apparent only at low doses, that are relatively specific for the $\alpha_2$-adrenergic receptor (9,11). These data are in partial agreement with those of Sara (20) who found that low (0.5-1.0 mg/kg) doses of yohimbine facilitate learning and retrieval of an appetitively motivated task. Sara found no locomotor activity differences in yohimbine-treated vs. control rats in the maze, but in her experiments yohimbine was given to animals that were already habituated to the apparatus. Our results suggest that yohimbine-induced arousal precedes effects on learning-related performance, and this action may not be apparent in habituated animals. It is also possible that the procedure used by Sara

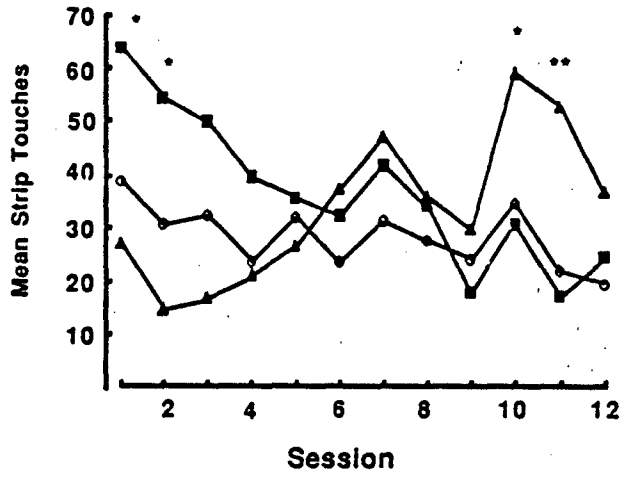


FIG. 3

Effect of yohimbine on unconditioned rearing (strip touching) activity. Open circles: water; dark squares: 0.5 mg/kg yohimbine; dark triangles: 1.5 mg/kg yohimbine. There was a Session by Treatment interaction ($F[22,198]=3.2$, $p<.001$). $^{*}p<.05$, $^{**}p<.01$ different from water.

was not sensitive to small increases in locomotor activity. Sara (20) has also found that amphetamine enhances performance in her task with no effects on activity, but we have found that a similarly small dose of this compound increases exploratory behavior in our apparatus (21).

Although the data support the notion that the arousal-inducing property of yohimbine precedes and is causally related to its learning-enhancing effects, it is possible that the former is not necessary. The fact that intertrial interval lever touches were significantly elevated in the high dose group, before showing an elevation in the control group, suggests that learning was occurring in this group, in spite of the fact that performance was suppressed until after discontinuation of treatment. Since high doses of yohimbine also block dopamine receptors in the CNS (9,11) motor retardation should be expected, and indeed it was observed during the first 6 sessions.

These results stand in apparent contrast to recent reports that the $\alpha_2$-adrenergic agonist clonidine facilitates learning and memory related performance in humans with Korsakoff psychosis (22) and in aged monkeys and monkeys with cortical norepinephrine depletion induced by 6-hydroxydopamine (23). However, clonidine had no effect in normal monkeys, and has been found to impair learning in normal humans (24). Yohimbine was found to have impairing effects at low doses in old monkeys, but only a relatively high (1.5 mg/kg) dose impaired performance in young monkeys (23), as in the present experiment. Thus, clonidine and yohimbine may exert paradoxical effects when functioning of central noradrenergic neurons is impaired: instead of

agonist-induced decreased norepinephrine release by direct action on autoreceptors or locus coeruleus, agonists may have their primary effects at postsynaptic $\alpha_2$-receptors in forebrain or other CNS sites (23). It remains to be determined whether low doses of clonidine also induce behavioral arousal and/or anxiety in subjects with central noradrenergic dysfunction, and whether this is related to its cognition-enhancing effects.

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## References

1. R.I. BLOCK and R. BERCHOU, Biochem. Behav. 20 233-241 (1984).
2. M. DAVIS, Psychopharmacology 62 1-7 (1979).
3. M.M. GHONEIM, J.V. HINRICHS and S.P. MEWALDT, Psychopharmacology 82 291-295 (1984).
4. R.A. JENSEN, J.L. MARTINEZ, JR., B.J. VASQUEZ and J.L. MCGAUGH, Psychopharmacology 64 125-126 (1979).
5. A. PLATEL and R.D. PORSOLT, Psychopharmacology 78 346-352 (1982).
6. P. VENAULT, G. CHAPOUTHIER, L. PRADO DE CARVALHO, J. SIMIAND, M. MORRE, R.H. DODD and J. ROSSIER, Nature 321 864-866 (1986).
7. S. CHARNEY, G.R. HENINGER and D.E. REDMOND, Life Science 33 19-29 (1983).
8. S.L. GARFIELD, S. GERSHON, I. SLETTEN, D.M. SUNDLAND and S. BALLOU, Int. J. Neuropsychiat. 3 426-433 (1967).
9. L.P. DWOSKIN, B.S. NEAL and S.B. SPARBER, Adv. Behav. Pharmacol. 6 45-81 (1986).
10. M.R. GOLDBERG and D. ROBERTSON, Pharmacol. Rev. 35 143-180 (1983).
11. B. SCATTON, B. ZIVKOVIC and J. DEDEK, J. Pharmacol. Exp. Therap. 215 494-499 (1980).
12. R.B. MESSING and S.B. SPARBER, Eur. J. Pharmacol. 89 43-51 (1983).
13. R.B. MESSING and S.B. SPARBER, Behav. Neurosci. 99 1114-1119 (1985).
14. L.W. CHANG and R.S. DYER, Neurobehav. Toxicol. Teratol. 5 673-696 (1983).
15. C.A. COHEN, R.B. MESSING and S.B. SPARBER, Psychopharmacology (in press).
16. R.B. MESSING, M.S. KLEVEN and S.B. SPARBER, Behav. Proc. 13 327-338 (1986).
17. L. LICHTBLAU and S.B. SPARBER, Neurobehav. Toxicol. Teratol. 4 557-565 (1982).
18. P.T. NINAN, T.M. INSEL, R.M. COHEN, J.M. COOK, P. SKOLNICK and S.M. PAUL, Science 218 1332 (1982).
19. D.E. REDMOND and Y.H. HUANG, Life Sci. 25 2149-2162 (1979).
20. S.J. SARA, Ann. N.Y. Acad. Sci. 444 178-193 (1985).
21. S.B. SPARBER, The Effects of Foods and Drugs on the Development of the Nervous System, p. 49-61, FDA, Rockville MD (1983).
22. W. MCENTEE and R.G. MAIR, Ann. Neurol. 27 466-470 (1980).
23. A.F.T. ARNSTEN and P.S. GOLDMAN-RAKIC, Science 230 1273-1276 (1985).
24. C.D. FRITH, J. DOWDY, I.N. FERRIER and T.J. CROW, Psychopharmacology 87 490-493 (1985).